Journal of Animal and Veterinary Advances 6 (6): 813-815, 2007

Polymorphism Prolactin Loci in Russian Cattle

[1]Masoud Alipanah, [2]Lobov Alexandrovna Kalashnikova and [3]Gennady Veladimirovich Rodionov
[1]Department of Animal Science, Zabol University, Zabol, 98516-538, Iran
[2]All-Russian Research Institute of Animal Breeding, Moscow, 141212, Russia
[3]Department of Diary Cattle Breeding,
Timiriazev Agricultural Academy, Moscow, 127550, Russia

Abstract: Genotypes of 72 Russian Black pied and 98 Red pied cows were determined for PRL-Rsa I loci by Restriction Fragment Length Polymorphism analysis (PCR-RFLP) of amplified DNA. This technique was used to determine the PRL-Rsa I allelic frequency in Black pied and Red pied dairy herds. Estimated gene frequencies were 0.71, 0.79 for the A and 0.29, 0.21 for the B alleles PRL-Rsa I for Black pied and Red pied breeds, respectively. There were no difference in the frequency of A and B alleles of Prolactin gene. A significant association between the PRL-Rsa I and milk yield, fat percentage is shown for two breeds.

Key words: Prolactin, polymorphism, milk, cattle, PRL, RFLP, Rsa

INTRODUCTION

PRL-Rsa I play an important regulatory function in mammary gland development, milk secretion and expression of milk protein genes. Hence the PRL gene is a potential genetic marker of production traits in dairy cattle. The gene was mapped on chromosome 23 by Hallerman *et al.* (1987). It consists of 5 exons and four introns (Camper *et al.*, 1984) encoding the 199-amino-acid mature protein. On the basis of sequence analysis of 4 different cDNA clones, seven possible nucleotide substitutions were described by Sasavage *et al.* (1974). One of them, recognized by *RsaI* endonuclease, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (Mitra *et al.*, 1995). Two allelic variants (*A* and B*)* have been distinguished at the DNA level, based on *RsaI* polymorphism in the third exon of the coding region. It has been suggested that PRL-Rsa I alleles correlate with milk yield (Lewin *et al.*, 1992).

The objectives of this research was to study gene frequencies at the PRL-Rsa I loci in Black pied and Red pied cattle and compare them with those reported for different commercial cattle breeds and to investigate the relationship between these polymorphisms and milk production traits of Black pied and Red pied cattle.

In Russia since 1977 began the work on the creation of new breed Red-Pied. The Red-Pied breed cattle was created via the crossing of Simmental cows with the bulls of the Red-Pied Holstein with the high milk yield and with fattiness 3,6- 3,7 %, the living mass of 600-650 kg, with the evenly developed udder, suitable for the machine milking.

The Black Pied breed developed from crossing the local cattle in various areas with the Dutch Black Pied and East Friesian breeds. Some animals were imported from Germany, the Netherlands, Estonia and Lithuania during 1930-40 and distributed in various parts of the Russia.

MATERIALS AND METHODS

A total of 72 Russian Black pied and 98 Russian Red Pied cows were genotyped. The cows were kept in the Gorki herd in Moscow state and in the Drydjba herd in Varonedj state of Russia, respectively.

Blood samples for DNA genotyping were collected from the jugular vein. The isolation of DNA from whole blood done with a method described by and Denicourt *et al.* (1990).

PRL-Rsa I polymorphism: The *PRL-RsaI* genotypes were analysed using the PCR-RFLP method. PCR Primer Sequences was amplified using forward 5'-CGAGTCCTTATGAGCTTGATTCTT-3' and reverse 5'-GCCTTCCAGAAGTC GTTTGTTTTC-3' primers (Mitra *et al.*, 1995). Cycles applied were: denaturation-94°C/5 min, followed by 30 cycles - 94°C/30 s, primer annealing- 59°C/40 s, PCR products synthesis - 72°C/20s and final synthesis-72°C/3 min. Amplified DNA was digested with *RsaI* enzyme. Digestion products were separated electrophoretically in 4% agarose gel. The polymerase chain reactions were performed using a

Corresponding Author: Masoud Alipanah, Department of Animal Science, Zabol University, Zabol, 98516-538, Iran

PCR-mix with: 2.5 μL 10 × PCR buffer (15 mM $MgCl_2$)1, 5 μL dNTP-mix (2 Mm each), 1.5 μL of primer (100 pmol/μL^{-1} each), 0/5 U Taq.

Allele frequencies were determined by gene counting. A Chi-square test was carried out to evaluate if the population was in Hardy-Weinberg equilibrium.

Data for 305-day milk production, including overall yields of milk, milk fat and milk protein, percent of milk fat and percent of milk protein obtained from the farm records. Milk samples were taken from each cow once a month during lactation, the milk samples from two control days were estimated for the contents of fat, protein on the basis of measurements by a milkoscan FT2 (Foss, Denmark) apparatus.

Statistical Analysis: The data obtained were analysis by the SAS procedure according to following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$$

Where:

Y_{ijkl} - Observed mean value of the trait;
μ - Overall mean;
a_i - Effect of i-th year of lactation (I = 1,…,6);
b_j - Effect of j-th lactation number (j = 1,…,6);
Ck - Effect of Prl-Rsa I genotype
(k - AA, AB and BB);
e_{ijkl} - Random error.

RESULTS AND DISCUSSION

In this study the frequencies of alleles PRL-Rsa I were as follows; A-0.71, B-0.29 for Black-Pied and A-0.79, B-0.21 for Red-Pied, respectively. The frequencies of AA, AB and BB genotypes were 0.500, 0.413 and 0.087 for Black-Pied 0.598, 0.392 and 0.01 for Red-Pied, respectively (Table 1).

Until now the frequency of PRL-Rsa I alleles in Russia has been analysed only for Russian Black-Pied. Frequencies of PRL-Rsa I alleles obtained in this study are similar to results reported earlier for the Russian Black-Pied cattle by Khatami that genotyped 32 cows and found that the allele frequencies for PRL-Rsa I A and B were 0.95 and 0.05, respectively. While was observed by Udina 0.80 and 0.20, for A and B alleles respectively. In other breeds higher frequency of the PRL-Rsa I A allele (0.95) in Holsteins was observed by Chrenek while lower by Mitra *et al.* (1995) 0.80 and 0.73, respectively. Dybus reported for Black and White cattle A-0.86 and B-0.14.

Effects of the PRL-Rsa I locus are shown in Table 2. In Black-Pied breed the effect of polymorphism PRL-Rsa I was significant for milk yield and fat percentage ($p = 0.05$). Milk of the cows with genotype BB of PRL-Rsa I contained more fat percentage (+0.19% and 0.12%, respectively) than AA and AB individuals. But AB genotype had higher milk yield (+ 356.37 kg and +761.17 kg, respectively) than AA and BB animals ($p = 0.05$).

Results in Red-Pied breed showed that cows with the BB genotype had higher milk yield, fat yield, protein yield but lesser fat percentage ($p = 0.05$). Animals with BB genotype and had higher milk yield and (+ 681.62 kg and + 906.73 kg, respectively) than AA and AB individuals. Milk fat yield and Differences ($p<0.05$) between the cows with different *PRL-Rsal* genotypes were observed. BB cows showed higher milk fat yield (+51.49 and +62.28 kg, respectively) than AA and A*B* individuals. *AA* cows yielded more milk fat (+10.79 kg) than *AB* animals. For milk fat content and (%).AB genotype and had higher fat content (+0.05 % and + 0.13%, respectively) than AA and BB individuals. For milk protein yield and the cows with the BB genotypes produced more milk protein (by 51.49 kg and 62.28 kg, respectively) than AA and A*B* individuals and cows with *AA* genotype produced more

Table1: Polymorphism at the K-casein and PRL-Rsa I loci in Russian breeds Black- pied and Red pied cattle

Breed	Genotype		Frequency	Allele (frequency)	χ^2
Black- Pied	PRL-	AA	500.00	A-0.71	0.02ns
	Rsa I	AB	41.30	B-0.29	
		BB	8.70		
Red Pied	PRL-	AA	59.79	A-0.79	0.14ns
	Rsa I	AB	39.18	B-0.21	
		BB	1.03		

Table 2: Effect of k-cas and PRL-Rsa I genotypes on the milk traits in Russian Black-Pied and Russian Red-Pied cows

Breed	Genotype	Milk(kg)±SE	Fat(kg)±SE	Fat(%)±SE	Protein(kg)±SE	Protein(%)±SE
Black-Pied	PRL-Rsa I					
	AA	7156.13±397.75^b	299.65±15.45	4.19±0.07^b	226.78±12.30^a	3.17±0.04^a
	AB	7512.50±397.75^a	311.00±15.45	4.16±0.07^b	233.71±12.30^a	3.12±0.04^a
	BB	6751.33±694.53^c	295.27±25.24	4.38±0.11^a	206.48±20.08^a	3.07±0.07^a
Red-Pied	PRL-Rsa I					
	AA	6136.90±178.03^b	236.46±8.81^b	3.73±0.04^a	236.46±8.81^b	3.27±0.03^a
	AB	5911.79±167.77^b	225.67±9.43^b	3.78±0.03^a	225.67±9.43^b	3.26±0.03^a
	BB	6818.52±576.92^a	287.95±31.03^a	3.65±0.12^b	287.95±31.03^a	3.27±0.11^a

^{a}within columns frequencies bearing the same superscripts differ significantly at $p = 0.05$

milk protein (+10.79 kg) than cows with *AB* genotype. Milk protein content and (%).No differences were found between the cows of different *PRL-Rsal* genotypes. The results shows that the highest milk and milk fat yield were obtained by cows with genotype *PRL-Rsal* BB. The results presented here show that the PRL-Rsa I gene may be considered as a marker for dairy traits in cattle.

CONCLUSION

Thus, it may be concluded that PRL-Rsa I genotypes, when used as genetic markers in selection programmes, may moderately, but significantly contribute to the improvement of milk production traits in cattle.

REFERENCES

Camper, S.A., N.N. Luck, Y.W. Yao, R.P. Ychik, R.G. Goodwin, R.H. Lyons and F.M. Rottman, 1984. DNA., 3: 237-249.

Denicourt, D., M.P. Sabour and A.J. Mcallister, 1990. Anim. Genet., 21: 215-216.

Hallerman, E.M., A. Nave and Y. Kashi, 1987. Anim. Genet., 18: 213-222.

Lewin, H.A., K. Schmitt, R. Hubert, M.J. Van Erik and N. Arnheim, 1992. Genomics, 13: 44-48.

Mitra, A., P. Schlee, C.R. Balakrishnan. and E. Pirchner, 1995. J. Anim. Breeding Genet., 112: 71-74.

Sasavage, N.L., J.H. Nilsson, S. Horowitz and F.M. Rotman, 1974. FEBS. Lett., 44: 205-208.